

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1 (original). A mixed sequence oligonucleotide comprising at least 12 nucleotides in length and having a 3' end and a 5' end and divided into a first portion and a further portion,

said first portion being capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide,

said further portion being incapable of supporting said cleavage by said RNase H1

wherein said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide.

Claims 2 to 28 cancelled.

Claim 29 (new). A mixed sequence oligonucleotide comprising at least 12 nucleotides and having a 3' end and a 5' end and divided into a first portion and a further portion,

said first portion supports cleavage of a complementary target RNA by a purified or isolated human RNase H1 polypeptide,

said further portion does not support said cleavage by said purified or isolated RNase H1 and where

said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide.

Claim 30 (new). The oligonucleotide of claim 29 comprising from about 12 to about 50 nucleotides.

Claim 31 (new). The oligonucleotide of claim 29 comprising from about 12 to about 25 nucleotides.

Claim 32 (new). A mixed sequence oligonucleotide comprising at least 12 nucleotides and having a 3' end and a 5' end and divided into a first portion and a further portion,

said first portion supports cleavage of a complementary target RNA by human RNase H1 polypeptide,

said further portion does not support said cleavage by said RNase H1;

wherein:

said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide;

each of said nucleotides of said first portion have B-form conformational geometry and are joined together in a continuous sequence, and

at least one of said nucleotides having B-form conformational geometry is not a 2'-deoxyribonucleotide.

Claim 33 (new). A mixed sequence oligonucleotide comprising at least 12 nucleotides and having a 3' end and a 5' end and divided into a first portion and a further portion,

said first portion supports cleavage of a complementary target RNA by human RNase H1 polypeptide,

said further portion does not support said cleavage by said RNase H1;

wherein:

said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide; and

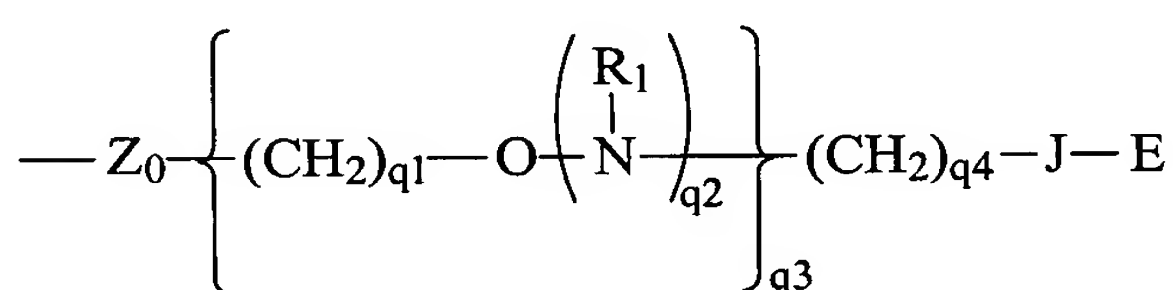
each of said nucleotides of said first portion is, independently, a 2'-SCH₃ ribonucleotide, a 2'-NH₂ ribonucleotide, a 2'-NH(C₁-C₂ alkyl) ribonucleotide, a 2'-N(C₁-C₂ alkyl)₂ ribonucleotide, a 2'-CF₃ ribonucleotide, a 2'=CH₂ ribonucleotide, a 2'=CHF ribonucleotide, a 2'=CF₂ ribonucleotide, a 2'-CH₃ ribonucleotide, a 2'-C₂H₅ ribonucleotide, a 2'-CH=CH₂ ribonucleotide or a 2'-C≡CH ribonucleotide.

Claim 34 (new). The oligonucleotide of claim 29 wherein at least one of said nucleotides of said first portion is a 2'-deoxyribonucleotide.

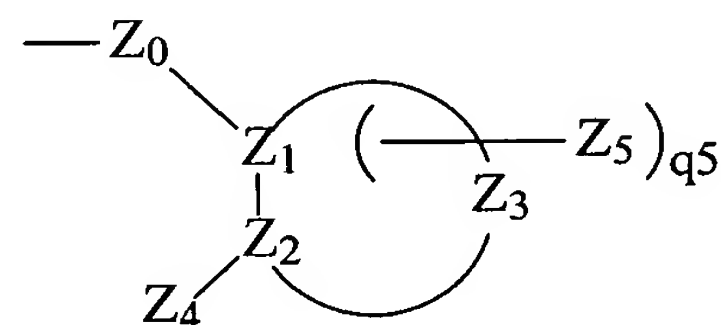
Claim 35 (new). The oligonucleotide of claim 29 wherein said nucleotides of said first portion are joined together in said continuous sequence by phosphorothioate linkages.

Claim 36 (new). The oligonucleotide of claim 29 wherein said further portion includes a plurality of nucleotides, at least some of said nucleotides comprise a 2' substituent group wherein each substituent group is, independently, hydroxyl, C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₂-C₂₀ alkynyl, halogen, amino, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, O-alkenyl, O-alkynyl, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or each substituent group has one of formula I or II:



I



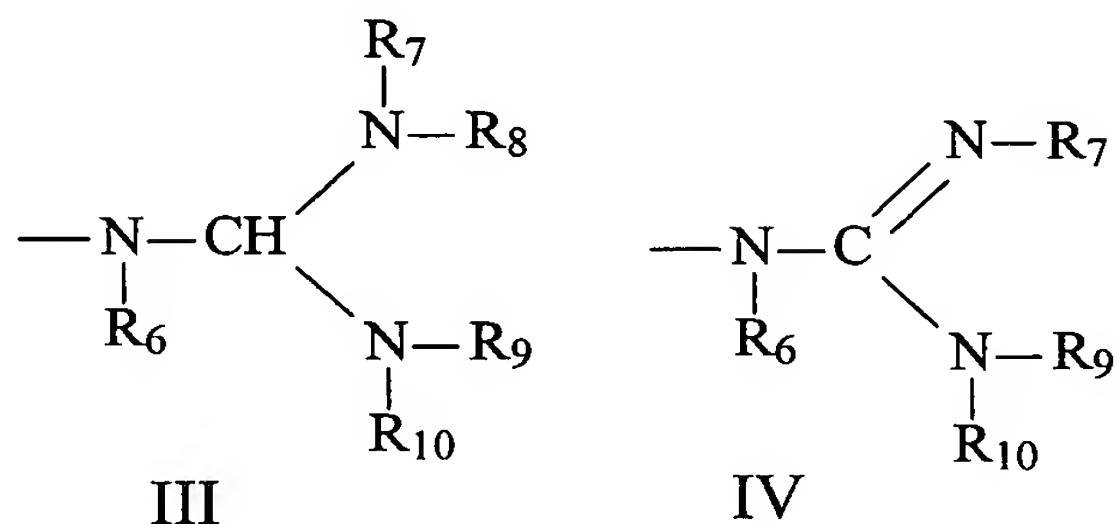
II

wherein:

Z₀ is O, S or NH;

J is a single bond, O or C(=O);

E is C₁-C₁₀ alkyl, N(R₁)(R₂), N=C(R₁)(R₂), or has one of formula III or IV;



each R_6 , R_7 , R_8 , R_9 and R_{10} is, independently, hydrogen, $C(O)R_{11}$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or optionally, R_7 and R_8 , together form a phthalimido moiety with the nitrogen atom to which they are attached;

or optionally, R_9 and R_{10} , together form a phthalimido moiety with the nitrogen atom to which they are attached;

each R_{11} is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

each R_1 and R_2 is, independently, H, a nitrogen protecting group, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, wherein said substitution is OR_3 , SR_3 , NH_3^+ , $N(R_3)(R_4)$, guanidino or acyl where said acyl is an acid amide or an ester;

or R_1 and R_2 , together, are a nitrogen protecting group or are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

or R_1 , T and L, together, are a chemical functional group;

each R_3 and R_4 is, independently, H, C_1 - C_{10} alkyl, a nitrogen protecting group, or R_3 and R_4 , together, are a nitrogen protecting group;

or R_3 and R_4 are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

Z_4 is OX, SX, or $N(X)_2$;

each X is, independently, H, C₁-C₈ alkyl, C₁-C₈ haloalkyl, C(=NH)N(H)R₅, C(=O)N(H)R₅ or OC(=O)N(H)R₅;

R₅ is H or C₁-C₈ alkyl;

Z₁, Z₂ and Z₃ comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

Z₅ is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, N(R₁)(R₂) OR₁, halo, SR₁ or CN;

each q₁ is, independently, an integer from 1 to 10;

each q₂ is, independently, 0 or 1;

q₃ is 0 or an integer from 1 to 10;

q₄ is an integer from 1 to 10; and

q₅ is from 0, 1 or 2;

provided that when q₃ is 0, q₄ is greater than 1.

Claim 37 (new). The oligonucleotide of claim 29 wherein each of said nucleotides of said further portion is, independently, a 2'-F ribonucleotide, a 2'-O-(C₁-C₆ alkyl) ribonucleotide, or a 2'-O-(C₁-C₆ substituted alkyl) ribonucleotide wherein the substitution is C₁-C₆ ether, C₁-C₆ thioether, amino, amino(C₁-C₆ alkyl) or amino(C₁-C₆ alkyl)₂.

Claim 38 (new). The oligonucleotide of claim 29 wherein said nucleotides of said further portion are joined together in a continuous sequence by 2'-5' phosphodiester, Sp phosphorothioate, Rp phosphorothioate, 3'-deoxy-3'-amino phosphoroamidate, 3'-methylenephosphonate, methylene(methylimino), amide 3, amide 4 or boranophosphate linkages.

Claim 39 (new). The oligonucleotide of claim 29 wherein at least two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion.

Claim 40 (new). The oligonucleotide of claim 29 wherein at least two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

Claim 41 (new). The oligonucleotide of claim 29 wherein at least two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion and at least two of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

Claim 42 (new). The oligonucleotide of claim 29 wherein at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion.

Claim 43 (new). The oligonucleotide of claim 29 wherein at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

Claim 44 (new). The oligonucleotide of claim 29 wherein at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion and at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

Claim 45 (new). A mixed sequence oligonucleotide comprising at least 8 nucleotides and having a 2'-OH arabinonucleotide sequence of at least 6 nucleotides and where at least one of said arabinonucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide, and wherein said oligonucleotide supports cleavage of a complementary target RNA by human RNase H1 polypeptide.

Claim 46 (new). A mixed sequence oligonucleotide comprising at least 8 nucleotides and having a 2'-F arabinonucleotide sequence of at least 6 nucleotides and where at least one of said 2'-F arabinonucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide, and wherein said oligonucleotide supports cleavage of a complementary target RNA by human RNase H1 polypeptide.

Claim 47 (new). A mixed sequence oligonucleotide comprising 8 to 25 nucleotides and having a 2'-OH arabinonucleotide sequence wherein at least one of the nucleotides of said sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide, wherein said oligonucleotide supports cleavage of a complementary target RNA by human RNase H1 polypeptide.

Claim 48 (new). A mixed sequence oligonucleotide comprising 8 to 25 nucleotides and having a 2'-F arabinonucleotide sequence wherein at least one of the nucleotides of said sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide, wherein said oligonucleotide supports cleavage of a complementary target RNA by human RNase H1 polypeptide.

Claim 49 (new). A chimeric oligonucleotide comprising 8 to 25 nucleotides and having a portion that supports cleavage of a complementary target RNA by human RNase H1 polypeptide wherein said portion supporting said cleavage is at least 6 nucleotides in length and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide, wherein said oligonucleotide supports cleavage of a complementary target RNA by human RNase H1 polypeptide.

Claim 50 (new). The oligonucleotide of claim 49 wherein said RNase H1 polypeptide is a purified or isolated polypeptide.

Claim 51 (new). A method comprising contacting an oligonucleotide according to claim 29 with RNA or DNA *in vitro*.

Claim 52 (new). A method comprising contacting an oligonucleotide according to claim 32 with RNA or DNA *in vitro*.

Claim 53 (new). A method comprising contacting an oligonucleotide according to claim 33 with RNA or DNA *in vitro*.

Claim 54 (new). A method comprising contacting an oligonucleotide according to claim 45 with RNA or DNA *in vitro*.

Claim 55 (new). A method comprising contacting an oligonucleotide according to claim 46 with RNA or DNA *in vitro*.

Claim 56 (new). A method comprising contacting an oligonucleotide according to claim 47 with RNA or DNA *in vitro*.

Claim 57 (new). A method comprising contacting an oligonucleotide according to claim 48 with RNA or DNA *in vitro*.

Claim 58 (new). A method comprising contacting an oligonucleotide according to claim 29 with RNA or DNA in a cellular assay.

Claim 59 (new). A method comprising contacting an oligonucleotide according to claim 32 with RNA or DNA in a cellular assay.

Claim 60 (new). A method comprising contacting an oligonucleotide according to claim 33 with RNA or DNA in a cellular assay.

Claim 61 (new). A method comprising contacting an oligonucleotide according to claim 45 with RNA or DNA in a cellular assay.

Claim 62 (new). A method comprising contacting an oligonucleotide according to claim 46 with RNA or DNA in a cellular assay.

Claim 63 (new). A method comprising contacting an oligonucleotide according to claim 47 with RNA or DNA in a cellular assay.

Claim 64 (new). A method comprising contacting an oligonucleotide according to claim 48 with RNA or DNA in a cellular assay.

Claim 65 (new). A method comprising contacting an oligonucleotide according to claim 49 with RNA or DNA *in vitro*.

Claim 66 (new). A method comprising contacting an oligonucleotide according to claim 49 with RNA or DNA in a cellular assay.

Claim 67 (new). A method comprising contacting an oligonucleotide according to claim 50 with RNA or DNA *in vitro*.

Claim 68 (new). A method comprising contacting an oligonucleotide according to claim 50 with RNA or DNA in a cellular assay.

Claim 69 (new). A method comprising:
selecting a parameter associated with interaction of an oligonucleotide, a target nucleic acid and human RNase H1 polypeptide,
selecting a cell containing human RNase H1 polypeptide and a target nucleic acid,
selecting first and second oligonucleotides each having a sequence substantially complementary to said target nucleic acid,
contacting said cell with said first oligonucleotide and measuring said parameter,
contacting said cell with said second oligonucleotide and measuring said parameter,
comparing said measurements, and
using said comparison to select one of said first or said second oligonucleotides for modulating said target in the presence of said human RNase H1 polypeptide in said cell.

Claim 70 (new). The method of claim 69 wherein said parameter is one of site preference for cleavage, sequence preference for cleavage or processivity of cleavage.

Claim 71 (new). The method of claim 69 wherein said measurement is a measurement of at least one of K_d , K_{max} , K_m or K_{cat} .

Claim 72 (new). An oligonucleotide for modulating a target nucleic acid in the presence of human RNase H1 polypeptide wherein said oligonucleotide is identified using the process of claim 69.

Claim 73 (new). An optimized oligonucleotide for modulating a target nucleic acid wherein said optimized oligonucleotide is identified using the process of claim 69.

Claim 74 (new). The method of claim 69 further comprising:
selecting a further oligonucleotide,
contacting said cell with said further oligonucleotide and measuring said parameter,

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comparing the measurement of this further oligonucleotide to that of said selected first or said second oligonucleotides and selecting one of said first, said second or said further oligonucleotide.